The Disappearance of Immuno-Reactive Insulin in Anephric Man and the Concomitant Effect on Glucose, Cortisol and Growth Hormone Levels

G SNODGRASS, R O ROBINSON, K MASHITER, C S OGG, J S CAMERON, F G ELLIS and L STIMMLER

Guy’s Hospital Medical School, London, United Kingdom

Impairment of carbohydrate tolerance in uraemic patients has been known for fifty years. This abnormality has been convincingly demonstrated following oral (Perkoff et al, 1958) and intravenous glucose loading (Horton et al, 1968) in such patients. This cannot be explained by a deficiency of insulin secretion, for plasma insulin levels have been shown to be relatively high and to remain elevated for an abnormal duration following oral (Tchobroutsky et al, 1965) or intravenous glucose loading (Hutchings et al, 1966). One explanation, currently favoured, is that of an increased tissue resistance to the normal effects of insulin (Cerletty & Engbring, 1967; Horton et al, 1968). It has been shown by Chamberlain and Stimmer (1967) that in normal subjects a significant difference exists (30%) between immunoreactive insulin (IRI) concentrations in renal venous and renal arterial blood. These workers assume an important role for the kidney for the degradation of insulin.

Using a different approach we have studied the disappearance of IRI in anephric subjects as compared with normal controls. We have also taken the opportunity to examine some aspects of function of the hypothalamic-pituitary-adrenal axis by measuring simultaneous changes in HGH and Cortisol levels during the same experimental situation.

METHODS

Fourteen normal volunteers (9 males and 5 females) and ten anephric patients (8 males and 2 females) were studied. The mean age of the normal controls was 35.5 years (range 24-63 years) and that of the anephric patients 32.4 years (range 20-56 years). The anephric patients were all having regular haemodialysis treatment for 14 hours twice weekly using KiiI dialysers with PT 300 cellulose membranes, at blood flows averaging 160 ml/min. Seven patients were studied immediately prior to dialysis and three studied two hours after cessation of treatment.

The study was begun after 14 hours of starvation in both normal and
anephric subjects. All subjects were rested for one hour prior to starting
the test. The mean pre-dialysis plasma creatinine was 15 mg/100 ml (SD
d + 5.6) for the anephric patients and the mean pre-dialysis blood urea was
177 (SD + 36) mg/100 ml. Post-dialysis the mean blood urea was 55.4
(SD + 25) mg/100 ml.

Fasting blood was taken for glucose, IRI, cortisol and human growth
hormone (HGH) levels. Porcine insulin, 0.1 u/kg body weight was then
given intravenously into the arm not being used for sampling. Further samples
were obtained from an indwelling venous cannula in the controls and from the
arterio-venous shunt in the anephric patients. Samples were then taken at
2½ minute intervals for 25 minutes, then at 5 minute intervals for a further 25
minutes and subsequently at 10 minute intervals for 30 minutes. All the
heparinised samples were kept on ice and separated in batches of five. Blood
glucose was measured by the glucose oxidase method using a standard auto-
analyser technique. Plasma IRI was estimated by the method of Morgan and
Lazarow (1962). Plasma HGH was also measured by a double antibody method
described by Hartog et al (1964). Plasma 11-hydroxycorticosteroids were
estimated by the method of Mattingly (1962) with slight modifications.

RESULTS

Insulin

The plasma levels of IRI before and after administration of porcine insulin
are shown for normal controls (Table I) and for the anephric patients (Table
II). Fasting values were similar for the two groups. Five minutes after

Table I. The plasma IRI in 14 normal adult subjects following
injection of porcine insulin 0.1 u/kg body weight

<table>
<thead>
<tr>
<th>Subject</th>
<th>Minutes 0</th>
<th>5</th>
<th>7.5</th>
<th>10</th>
<th>12.5</th>
<th>15</th>
<th>17.5</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
<th>45</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>910</td>
<td>305</td>
<td>182</td>
<td>120</td>
<td>94</td>
<td>65</td>
<td>49</td>
<td>29</td>
<td>21</td>
<td>13</td>
<td>9</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>397</td>
<td>310</td>
<td>199</td>
<td>152</td>
<td>132</td>
<td>72</td>
<td>49</td>
<td>30</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>240</td>
<td>229</td>
<td>188</td>
<td>131</td>
<td>88</td>
<td>70</td>
<td>58</td>
<td>35</td>
<td>21</td>
<td>13</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>460</td>
<td>363</td>
<td>153</td>
<td>107</td>
<td>89</td>
<td>72</td>
<td>57</td>
<td>39</td>
<td>30</td>
<td>21</td>
<td>12</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>415</td>
<td>260</td>
<td>151</td>
<td>69</td>
<td>64</td>
<td>41</td>
<td>30</td>
<td>18</td>
<td>11</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>300</td>
<td>240</td>
<td>48</td>
<td>57</td>
<td>67</td>
<td>54</td>
<td>41</td>
<td>14</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>700</td>
<td>425</td>
<td>245</td>
<td>183</td>
<td>135</td>
<td>119</td>
<td>91</td>
<td>60</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>745</td>
<td>500</td>
<td>378</td>
<td>200</td>
<td>130</td>
<td>85</td>
<td>40</td>
<td>25</td>
<td>50</td>
<td>20</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1700</td>
<td>970</td>
<td>330</td>
<td>230</td>
<td>180</td>
<td>130</td>
<td>80</td>
<td>50</td>
<td>40</td>
<td>20</td>
<td>12</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>450</td>
<td>445</td>
<td>195</td>
<td>157</td>
<td>110</td>
<td>100</td>
<td>90</td>
<td>65</td>
<td>0</td>
<td>15</td>
<td>30</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>2930</td>
<td>1545</td>
<td>200</td>
<td>190</td>
<td>0</td>
<td>30</td>
<td>27</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1510</td>
<td>500</td>
<td>475</td>
<td>370</td>
<td>190</td>
<td>135</td>
<td>105</td>
<td>100</td>
<td>65</td>
<td>15</td>
<td>35</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>700</td>
<td>580</td>
<td>400</td>
<td>190</td>
<td>150</td>
<td>90</td>
<td>45</td>
<td>65</td>
<td>80</td>
<td>0</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>980</td>
<td>837</td>
<td>470</td>
<td>330</td>
<td>200</td>
<td>163</td>
<td>120</td>
<td>67</td>
<td>43</td>
<td>34</td>
<td>23</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>7 867</td>
<td>519</td>
<td>280</td>
<td>183</td>
<td>119</td>
<td>90</td>
<td>68</td>
<td>42</td>
<td>28</td>
<td>19</td>
<td>13</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

226
Table II. The plasma IRI in 10 anephric patients following porcine insulin 0.1 u/kg body weight

<table>
<thead>
<tr>
<th>Subject</th>
<th>Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td>9</td>
</tr>
</tbody>
</table>

Mean: 8 399 321 213 167 121 83 75 64 55 41 28 22

Figure 1. The mean IRI disappearance curve for normal controls and anephrics

intravenous insulin 0.1 u/kg body weight the levels were significantly lower in the anephric cases. Analysis of the IRI values from 5-20 minutes following insulin administration can be used to calculate its disappearance rate from plasma. The mean plasma disappearance rate of IRI for normals was found to be 17 ± 6% (SD) per minute, for anephrics it was 11 ± 1% (SD) per minute. The difference between the two groups was significant (t = 4.37, p < 0.0005). Although the mean 5 minute level of IRI is lower in the anephric group, it is the same at 15 minutes (Figure 1) and thereafter exceeds it. This is explained by the slower disappearance of IRI in anephric patients.
Glucose

There had already been a fall in the blood glucose levels 5 minutes after insulin administration in both groups. From 5-20 minutes after the insulin this fall was exponential (Figure 2). The mean rate of blood glucose disappearance for normals was $7.2 \pm 2.6\%$ (SD) per minute and $3.5 \pm 1.4\%$ (SD) per minute for anephric cases. This difference was significant ($t = 3.28$, $p < 0.0025$). The point at which the blood glucose reached its nadir was $27 \pm 2.7$ (SD) minutes for normals and $38.5 \pm 14$ minutes (SD) for the anephrics ($t = 2.53$, $p = 0.01$). The degree of the glucose fall was only slightly greater in the normal subjects than in the anephric patients. Expressed as a percentage of the fasting level this was 75% at the mean nadir for the controls and 63% at the mean nadir for glucose for the anephric cases.

![Figure 2. The mean glucose disappearance for normals and anephrics expressed as a percentage of the fasting level](image)

Growth hormone

The mean plasma HGH values for the controls and for the anephrics are shown in Figure 3. The mean fasting levels were $5 + 3$ (SD) ng/ml for anephric patients and $4 + 3$ (SD) ng/ml for the controls. As can be seen these are very similar. There was a marked difference found for the peak values achieved, however. For normal subjects this was $36 + 10$ (SD) ng/ml while for the anephrics it was $53 + 18$ (SD) ng/ml. This difference was significant ($t = 2.28$, $p = 0.025$). Peak values also occurred earlier in the anephric group ($39 + 9$ minutes) than in the normal controls ($52 + 16$ minutes) and this difference was also significant ($t = 2.17$, $p = 0.025$).

Cortisol

The mean resting cortisol levels in the anephric group was $21 + 5$ (SD) $\mu$g/100 ml. This is rather high, but is compatible with the values obtained in a normal individual under conditions of stress. At 30 minutes the mean level
was 24 ± 11 (SD) μg/100 ml and at 60 minutes 33 ± 11 (SD) μg/100 ml. There was a satisfactory rise found, therefore, in response to the hypoglycaemic stress as reflected in the 60 minute values.

DISCUSSION

Although fasting levels of IRI were comparable for the two groups and equivalent doses of insulin had been given to all subjects according to body size, the 5 minute values were significantly lower in the anephric group (t = 1.9, p < 0.05). These low values can be partially explained by the increased plasma volumes of these patients, resulting in a greater initial dilution of the administered insulin. The anephric patients were anaemic (mean haemoglobin 7.3 ± 1.1 (SD) g/100 ml; PCV 22 ± 4 (SD) %). Several explanations have been offered for the slower disappearance of exogenously administered insulin in uraemia. These include a generalised tissue unresponsiveness to the action of insulin or a defect in its binding to sites of action in the tissues (Horton et al, 1968). If unresponsiveness of the tissues is the cause then it is not due to urea retention alone (Horton et al, 1968) and this is supported in the present study by finding no difference in the IRI disappearance rate for those cases studied before dialysis and those studied after dialysis. For the mean post-dialysis blood urea was 55.4 ± 25 mg/100 ml in these patients as compared with a mean level of 177 ± 36 (SD) during the same experimental situation for those tested before dialysis.

The important role of the kidney in man for insulin degradation has been shown by Chamberlain and Stimmler (1967). These authors have shown that 30% of the IRI passing through the kidneys is removed by them. Assuming
that 20% of the cardiac output passes through the kidneys it can be calculated
that the renal rate of removal would amount to 6% per minute. From the data
given it can be seen that the difference in the IRI disappearance rates between
the two groups could therefore be explained by the absence of kidneys alone.

Within five minutes of insulin administration there was already an obvious
effect on blood glucose levels. The rapidity of this effect is likely to be due
to the high concentrations of circulating insulin present at this time, for as
insulin levels fall so the rate of glucose disappearance falls until the nadir
for glucose is reached. The later occurrence of the glucose nadir in anephric
patients could be explained by the persistence of the exogenous insulin in their
plasma for a longer period. That is to say, the insulin in the plasma contin-
ues to exert an effect on the blood glucose until it has disappeared. It can be
calculated that when the IRI has fallen to 30 μu/ml then approximately 99%
of the administered dose has disappeared from the plasma. The mean IRI
at the nadir for glucose was 34 μu/ml for the normals and 29 μu/ml for the
anephrics. An alternative explanation for the delayed glucose nadir in ane-
phrics might be that the homeostatic mechanisms responsible for restoration
of normal glucose levels are inadequate or delayed in their effect. The sub-
stantial and significantly early HGH peak levels, together with a highly ade-
quate Cortisol response which is normal in timing and type, would make this
suggestion unlikely. The lower rate of glucose disappearance in anephrics
could be explained by the absence of kidneys which, under the influence of
adequate amounts of circulating insulin, might be expected to take up a pro-
portion of the glucose passing through them.

The significantly higher peak values of HGH in anephric patients would
appear to be due to an increased secretion, rather than to a delay in its
degradation, for a normal pattern of fall is seen subsequently. This fall has
reached near basal levels at the 80 minute point of the test. The fasting
values and those occurring up to 17.5 minutes after insulin are extremely
similar for the two groups. Subsequent to this time a rapid rise in HGH
occurs in the anephric group. The significantly earlier occurrence of the
HGH peak in anephrics is not associated with an earlier glucose nadir, how-
ever. Protein-calorie malnutrition has been found to be associated with high
fasting levels of HGH (Pimstone et al, 1967), but these patients averaged 55g
protein/day, predominantly of animal origin, and their fasting HGH values
were comparable with those of the normal controls.

The Cortisol response to hypoglycaemia showed that no defect of the
hypothalamic-pituitary-adrenal axis was present. Adrenal reserve for Cort-
isol was also normal.
ACKNOWLEDGMENTS

The plasma Cortisol estimations were performed by Dr John Townsend and Dr Janice Went of the Department of Clinical Pathology at Guy's Hospital. The blood glucose were also performed by this Department with the kind permission of Dr J Liddell.

REFERENCES

Hartog, M., Gaafar, M. A., Meisser, B. and Fraser, R. (1964) British Medical Journal, 2, 1229
Horton, E. S., Johnson, C. and Lebowitz, H. E. (1968) Annals of Internal Medicine, 68, 63